Synchronizing neurotransmission

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1. Summary

The aim of this thesis was to examine presynaptic mechanisms underlying modulation of synaptic strength and release kinetics. We first explored the impact of changing the energy requirements for fusion. Second we studied supralinear Ca^{2+}-sensitivity in the context of an energy barrier model, and whether Ca^{2+}-binding to Synaptotagmin-1 (Syt1), the main Ca^{2+}-sensor for release, contributes to decreasing the fusion energy barrier. Third, we explored interplay between separate factors in reducing the energy barrier to collaboratively increase synaptic strength in a multiplicative way. Fourth, we examined the energy barrier dependent and independent contributions from the DAG and PKA pathways to potentiation of release. Finally, we investigated how Syt1's release inhibitory function may contribute to maintaining high-frequency synchronous release, by controlling the activation of a second Ca^{2+}-sensor.

In **chapter 2** we presented a vesicle state model capable of fitting hypertonic sucrose responses at different concentrations. This method yields estimates of the readily releasable pool (RRP), vesicle priming, unpriming, and fusion rate constants. Using the Arrhenius equation, changes in vesicle fusion rates can be represented as changes in the fusion energy barrier. We demonstrated that separate modulators of synaptic vesicle release act additively on the energy barrier, resulting in multiplicative effects on fusion rates. Furthermore, we hypothesized that such an energy barrier model for vesicle fusion could underlie the supralinear Ca^{2+}-sensitivity of release, as described by the allosteric model for Ca^{2+}-dependent release [1].

In **chapter 3**, we explored this hypothesis experimentally and showed that Syt1 contributes to vesicle fusion by lowering the energy barrier with its Ca^{2+}-binding C2A domain. We also showed that the release inhibitory function of Syt1 is independent of energy barrier modulation. Next, we presented an extension to the model with a second and slower sensor. Several factors may lower the energy barrier independently, yet produce multiplicative effects on release rates. Therefore, a dual sensor model not only adds a second asynchronous release pathway, but also potentiates synchronous release when both sensors are activated simultaneously. We provided experimental support for such a scheme by showing that activation of the DAG-pathway, which potentiates synchronous release, lowers the energy barrier both in the presence and absence of Syt1, presumably by activation of a second sensor.

In **chapter 4**, we further investigated the inhibitory function of Syt1 and its effects on the synchronicity of release. We showed that Syt1's release inhibitory function is strongly temperature dependent. Furthermore, by suppressing asynchronous release, Syt1's inhibitory function plays a

decisive role in maintaining sufficient synchronously releasable vesicles to sustain high-frequency synchronous release.

Finally, in **chapter 5** we explored potentiation of presynaptic release induced by the PKA-pathway. We found that activation of PKA preferentially boosts low release probability synapses, in part by lowering the fusion energy barrier. Furthermore, PKA-activation can restore large portions of synaptic transmission in priming deficient synapses. We presented evidence for the Thr138 phosphorylation site in SNAP25 as a relevant target for PKA mediated potentiation, addressing a long standing question in the field.

Overall, we conclude that modulation of the energy barrier is a relevant mechanism in synchronous release triggering, presynaptic short-term plasticity (STP). Energy barrier dependent and independent mechanisms may cooperate to dynamically control synaptic vesicle release, synaptic transmission and -plasticity.